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Poster Session 9: Authophagy and intracellular trafficking

PS9-1: Moderate overexpression of *SEC16* improves α-Amylase secretion in *Saccharomyes cerevisiae*

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There is a large and increasing demand of recombinant proteins, not only for pharmaceuticals but also in the field of industrial enzymes . Recombinant proteins can be produced by a range of different hosts, , including mammalian cells, insect cells, bacteria, yeasts and fungi . Each of these have their own advantages and disadvantages, and none would naturally make a suitable general platform for producing of a wide range of recombinant proteins, with satisfactory yield, titer and productivity. Therefore, host optimization is required for the development of efficient recombinant protein producers. Yeast *Saccharomyces cerevisiae* is one of preferred model microbial and eukaryal systems, because of its robustness, well-studied genetics and physiology, developed molecular tools and large free databases. The limitations sometimes include translation and sometimes the folding and secretory capacity, which is more challenging to address. In this study, we focused on the secretory pathway and improved the secretion of a model enzyme α-amylase by overexpressing *SAR1*, which is the trigger of COPII vesicle formation, and *SEC2*, *SEC4*, *SEC15* and *YPT32*, which are required for Golgi-derived vesicle budding and transport. When these secrcetory proteins were overexpressed individually from a low copy number plasmid with the strong constitutive promoter TEF, only *SEC4* overexpression strain showed ~ 20% improvement in the final titer of α-amylase. The result indicates that overexpression of secretion-related proteins individually might not have a huge improvement on the production of α-amylase, so we will focus on the combination of these secretion-related proteins.
[1] Hou, J., et al. (2012) *FEMS Yeast Res* 12**,** 491-510; [2] Huang, M., et al. (2014). *Pharm. Bioprocess* **2**(2): 167-182.

PS9-2: Genetic control of the inactivation and degradation of the cytosolic proteins in methylotrophic yeast

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Methylotrophic yeasts are capable to metabolize one-carbon compound methanol as sole carbon and energy source. Many enzymes of methanol metabolism are located in peroxisomes whereas some of them are of a cytosolic localization. Shift of methanol-grown cells into a glucose-containing medium leads to fast inactivation of peroxisomal and cytosolic enzymes of methanol metabolism. Inactivation of peroxisomal enzymes occurs due to the autophagic degradation (pexophagy) whereas mechanisms of the inactivation of cytosolic enzymes like fructose-1,6-bisphosphatase (FBPase), formaldehyde and formate dehydrogenases remain unknown. In baker's yeast, the catabolite degradation of FBPase occurs after shift of glucose-starved cells into a glucose-containing medium. It was shown that FBPase is degraded by the proteasome-dependent pathway after glucose starvation of the yeasts for 1 day and by the vacuole-dependent pathway (autophagy) after glucose starvation of the cells for 3 days. We studied mechanisms of FBPase degradation in methylotrophic yeasts. The wild type strain of *Pichia pastoris* GS200, the protease-deficient strain SMD1163 (*pep4, prb1*) and the strain with deletion of a gene of a glucose sensor, Gss1p were used in this research. FBPase activity and protein amount was studied after shift of methanol-grown cells into a glucose medium with proteasome inhibitor MG132 and without it. Substantial decrease of the specific activity of FBPase in the wild-type strain and strain defective in vacuolar proteases and the minor change of the activity in the *Δgss1* strain in the cells without the inhibitor was observed. We also compared the FBPase activity of the strains defected in autophagy pathway (Δ*mon1,* Δ*ypt7,* Δ*ccz1*) with the wild type strain of *P. pastoris*. The results of Western blot analysis showed decrease in FBPase quantity in the GS200 strain and the minor decrease this protein in the SMD1163 strain after transfer of cells from methanol medium in